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Inversion of Sucrose by Invertase and the Separation of Fructose and Glucose by Ion Exchange Chromatography

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INVERSION OF SUCROSE BY INVERTASE AND THE SEPARATION OF FRUCTOSE AND GLUCOSE BY ION EXCHANGE CHROMATOGRAPHY

by

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"Inversion of Sucrose by Invertase and the Separation of Fructose and Glucose by Ion Exchange Chromatography"

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Foremost, I would like to thank my advisor Dr. James T. Hsu for his instrumental support and valuable advice that led to the completion of this thesis. I'm grateful for his constructive criticism based on his extensive knowledge and industry experience. I always enjoy our conversations, whether they be related to current events or a number of other topics; just as in research, there is always more to a story.

I would especially like to thank my parents for all their help and support throughout this challenging work. Also, to Ben Schwartz for his assistance with data collection and analysis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The primary goal of this thesis is to present a fundamental understanding of ion exchange chromatography and enzyme kinetics. Both techniques are explored through simple examples such as the inversion of sucrose by invertase as well as the separation of fructose and glucose by ion exchange chromatography. Enzymes are biocatalysts that allow biological systems to function, which can also be utilized for a variety of industrial reactions. Ion exchange chromatography is widely implemented in the pharmaceutical and biotechnology industries as well as by environmental industry, or for water filtering by consumers in their homes.

The hydrolysis of sucrose is catalyzed by the enzyme invertase. An enzymatic assay was used to determine the Michaelis-Menten constant and the maximum reaction rate. Initial reaction rates were determined by plotting glucose concentration versus time. These rates were then used to create a double reciprocal, or Lineweaver Burke, plot to determine the Michaelis-Menten constant and the maximum reaction rate. The Michaelis-Menten constant of invertase was found to be 9.97 mM and the maximum velocity was found to be 17.57 mMol/(L*min).

Ca-Cation exchange chromatography is useful for separating mixtures of glucose and fructose. The basis of separation is the stronger interaction between fructose and the calcium ion complex than glucose and the calcium ion complex. Physical parameters such as temperature and pH were varied to determine their effects on separation. The data show that lower temperatures increase the retention time of fructose and do not affect the retention time of glucose.

CHAPTER 1

INVERSION OF SUCROSE BY INVERTASE

In the presence of invertase, sucrose converts to fructose and glucose. This hydrolysis reaction also occurs in acidic conditions [1]. The objective of this work, however, is to study the reaction kinetics of invertase using an enzymatic assay. The concentration of glucose is measured over time for different concentrations of sucrose. Sodium carbonate, Na_2CO_3, is used to stop the reaction and make the solution slightly alkaline. This reaction is expected to follow Michaelis-Menten kinetics, which allows for simpler calculation.

1.1 Introduction

The purpose of this experiment is to study the reaction kinetics of invertase by determining the Michaelis-Menten constant as well as the maximum velocity. The Michaelis-Menten constant, K_M, is the substrate concentration required to fill half of the active sites of an enzyme and achieve V_{Max} [2]. While V_{Max}, the maximum velocity, describes the specific point in an enzymatic reaction at which the rate of the reaction is catalyzed to the maximum[2]. K_M is an inverse measure of affinity; a high K_M correlates to the enzyme having a low affinity for its substrate and therefore requiring a higher substrate concentration to achieve V_{Max}. Knowing the values of K_M and V_{Max} is useful for understanding enzyme activity and the effects of different types of enzyme inhibitors. This experiment studies invertase, which catalyzes the hydrolysis of sucrose to form an equimolar mixture of the invert sugars fructose and glucose. This reaction is expected to follow Michaelis-Menten kinetics, which is demonstrated in this lab using an enzymatic assay. Equation 1.1 depicts

the two step reaction mechanism predicted by the Michaelis-Menten model,

$$\mathrm{E} + \mathrm{S} \underset{\mathrm{k_2}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} [\mathrm{ES}] \xrightarrow{\mathrm{k_3}} \mathrm{E} + \mathrm{P} \tag{1.1}$$

where E is the enzyme, S is the substrate, [ES] is the enzyme-substrate complex, and P is the product [2]. Figure 1.1 depicts the overall reaction mechanism.

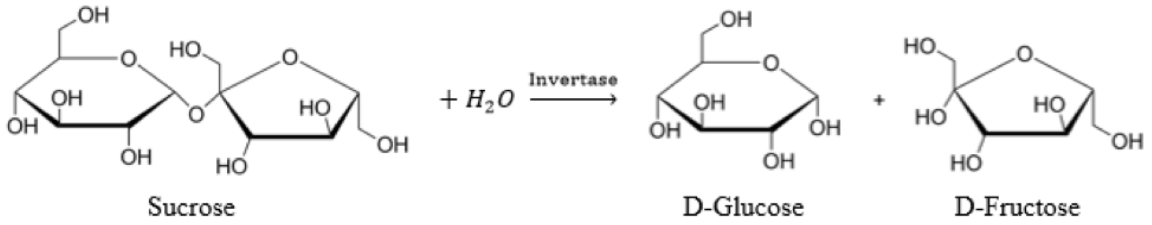

Figure 1.1: *Reaction mechanism for the hydrolysis of sucrose catalyzed by invertase*

The rate expression for this reaction is shown as Equation 1.2

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \tag{1.2}$$

where V_0 is the initial reaction rate in mMol/liter-min, [S] is the substrate concentration in mMol/liter, K_M is the Michalis-Menten constant in mM, and V_{Max} is the maximum reaction rate in mMol/liter-min[2]. One mole of sucrose reacts to form one mole of glucose and one mole of fructose as shown in Figure 1.1. Therefore, the initial rate of reaction can be experimentally determined by plotting the glucose concentration versus time instead of the sucrose concentration. Initially, the curve is linear; the slope of this linear section, shown as Equation 1.3, is the initial reaction rate

$$V_0 = \frac{d[Glucose]}{dt}\Big|_{t=0} = -\frac{d[S]}{dt}\Big|_{t=0} \tag{1.3}$$

where [Glucose] is the concentration of glucose in mMol/L determined using a VacciXCell GlucCell (Singapore) glucose analyzer and [S] is the substrate, sucrose, concentration. The reaction is stopped using sodium carbonate, Na_2CO_3, by making the solution slightly

alkaline and deactivating the enzyme. Repeat experiments are done to find initial rates of reaction for various sucrose concentrations. A Lineweaver Burke plot is made by plotting $1/V_0$ versus 1/[S]. Equation 1.4 can be used to solve for K_M and V_{Max} knowing the slope of the line is equal to $\frac{K_m}{V_{max}}$ and its intercept is equal to $\frac{1}{V_{max}}$.

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \tag{1.4}$$

1.2 Experimental Methods

30 mL of 12 mg/mL sucrose solution in DI water was made as a stock solution. The other sucrose concentrations (6 mg/mL and 3 mg/mL) were made by diluting the stock solution. Additionally, 50 mL of 1M Na_2CO_3 in DI water was created and used as a stop solution for the reaction after incubation of the samples. Finally, 10 mL of 1.5 mg/mL of invertase in DI water was created and used as the enzyme for the hydrolysis reactions.

For the assay, 1.5 mL of the sucrose solution was added to seven 13mm test tubes and then 0.1 mL of the 1.5 mg/mL enzyme solution was added to each test tube, mixed, and allowed to react for a predetermined amount of time. Refer to Table 1.1 for specific time intervals. After the time interval, 1.4mL of the 1M Na_2CO_3 solution was added and immediately vortexed to stop the reaction of sucrose to fructose and glucose. The concentration of glucose was then measured via a using a VacciXCell GlucCell (Singapore) glucose analyzer. This method was then repeated for sucrose concentrations of 6 mg/mL and 3 mg/mL at constant enzyme concentration of 1.5 mg/mL. Results were analyzed to determine Michaelis-Menten parameters as well as the accuracy of the experiment based on literature values of the specific activity of invertase. The materials and equipment used in this lab are listed in Appendix A in Table A.2 and Table A.1 respectively.

1.3 Results

The concentration of glucose was measured after allowing the reaction to proceed for a predetermined amount of time. Figure 1.2 depicts the concentration of glucose, after correcting for the dilution of the stop solution, of solutions with different starting sucrose concentrations at times ranging from 10 seconds to 1200 seconds.

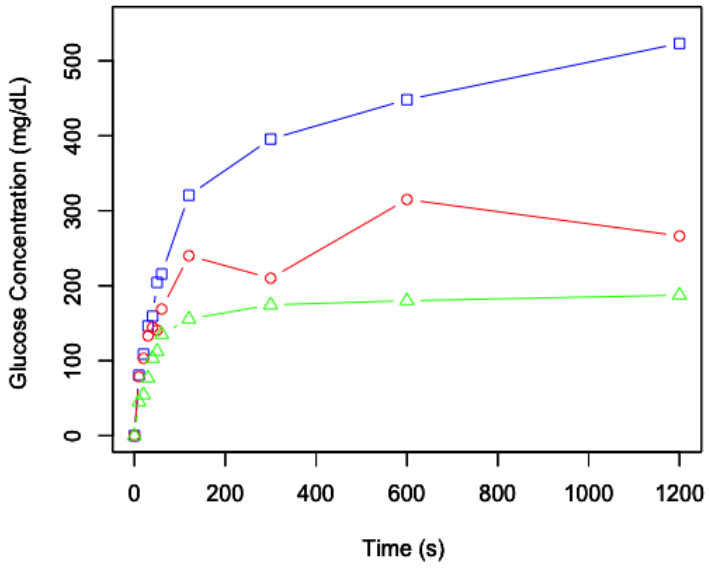

Figure 1.2: *Concentration of glucose (mg/dL) as a function of time (s) for solutions with varied sucrose concentrations and a constant enzyme concentration of 1.5mg/mL. Experimentally measured values are plotted in blue squares (12 mg/mL), red circles (6 mg/mL), and green triangles (3 mg/mL).*

Adding the stop solution to the test tube dilutes the concentration therefore Equation 1.5 is used to determine the corrected glucose concentration

$$C_1 * V_1 = C_2 * V_2 \tag{1.5}$$

where C_1 is the measured glucose concentration in mg/dL, V_1 is the total volume of the test tube including the stop solution equal to 3.0 mL, C_2 is the corrected glucose concentration in mg/dL and V_2 is the initial volume before the stop solution was added equal to 1.6 mL. Equation 1.5 can then be simplified to Equation 1.6.

$$C_2 = C_1 * 1.875 \tag{1.6}$$

this concentration is then converted into mMol/L using Equation 1.7

$$C_3 = \frac{C_2}{18} \tag{1.7}$$

where C_3 is the glucose concentration in mMol/L and X is the glucose concentration in mg/dL. Table 1.1, Table 1.2, and Table 1.3 depicts the same data shown in Figure 1.2 as well as the glucose concentration converted into mM/L.

Table 1.1: *Glucose Concentration vs. Time (12 mg/mL Sucrose)*

Time (s)	Glucose Concentration (mg/dL)	Corrected Glucose Concentration (mg/dL)	Glucose Concentration (mMol/L)
10	43	80.63	4.48
20	58	108.75	6.04
30	78	146.25	8.13
40	85	159.38	8.85
50	109	204.38	11.35
60	115	215.63	11.98
120	171	320.63	17.81
300	211	395.3	21.98
600	239	448.13	24.90
1200	279	523.13	29.06

Table 1.2: *Glucose Concentration vs. Time (6 mg/mL Sucrose)*

Time (s)	Glucose Concentration (mg/dL)	Corrected Glucose Concentration (mg/dL)	Glucose Concentration (mMol/L)
10	42	78.75	4.38
20	55	103.13	5.73
30	71	133.13	7.40
40	77	144.38	8.02
50	75	140.63	7.81
60	90	168.75	9.38
120	128	240.00	13.33
300	112	210.00	11.67
600	168	315.00	17.50
1200	142	266.25	14.79

Table 1.3: *Glucose Concentration vs. Time (3 mg/mL Sucrose)*

Time (s)	Glucose Concentration (mg/dL)	Corrected Glucose Concentration (mg/dL)	Glucose Concentration (mMol/L)
10	24	45.00	2.50
20	29	54.38	3.02
30	41	76.88	4.27
40	55	103.13	5.73
50	60	112.50	6.25
60	72	135.00	7.50
120	83	155.63	8.65
300	93	174.38	9.69
600	96	180.00	10.00
1200	100	187.50	10.42

The results summarized in Table 1.1, Table 1.2, and Table 1.3 are used to graphically determine the initial velocities of each concentration, shown as d[Glucose]/dt in Equation 1.3. There is a linear region, the slope of which is equal to the initial rate of reaction. Figure 1.3 depicts the initial reaction rates for varied starting sucrose concentrations.

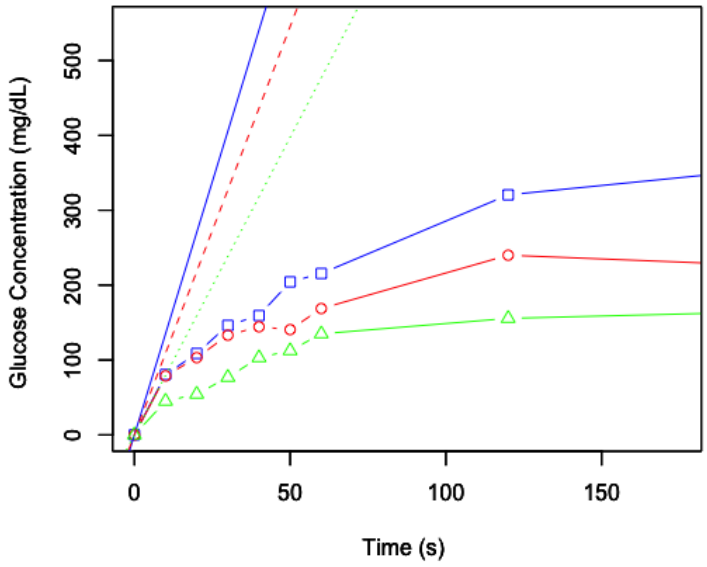

Figure 1.3: *Glucose concentration (mMol/L) as a function of time (min). Experimentally measured values for initial sucrose concentrations of 12 mg/mL, 6 mg/mL and 3 mg/mL are plotted in blue squares, red circles, and green triangles respectively. The linear fits for 12 mg/mL, 6 mg/mL and 3 mg/mL initial sucrose concentration are plotted as a solid blue line, dashed red line, and dotted green line respectively.*

The concentration of sucrose must also be corrected, as adding the enzyme solution dilutes the concentration slightly using Equation 1.5. Table 1.4 summarizes the corrected sucrose concentration as well as the initial slope values.

Table 1.4: *Experimentally determined initial velocities*

Concentration of Sucrose (mg/mL)	Concentration of Sucrose (mMol/L)	Initial rate d[Glucose]/dt (1/min)	1/[S] (L/mMol)	1/Vo (min)
2.81	8.21	7.94	0.12	0.13
5.63	16.43	10.91	0.06	0.09
11.25	32.87	13.52	0.03	0.07

Plotting the inverted reaction velocity as a function of the inverse of sucrose concentration produces a double reciprocal plot, also know as a Lineweaver-Burke plot. Figure 1.4 depicts this plot using the data found in Table 1.4.

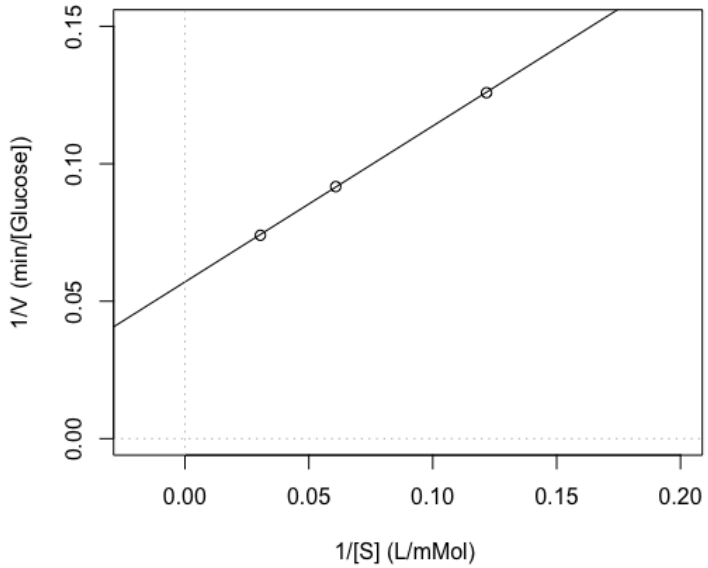

Figure 1.4: *Lineweaver Burke Plot. Inverse sucrose concentration (L/mMol) as a function of inverted initial reaction rate (min/[Glucose]) Note: [Glucose]=[Sucrose]. Experimental values plotted as black circles and a linear fit plotted in black.*

The slope and Y-intercept of the line in Figure 1.4 are used to calculate K_m and V_{max} using the Michaelis-Menten Equation shown as Equation 1.4. The slope is equal to $\frac{K_m}{V_{max}}$, whereas the Y-intercept is equal to $\frac{1}{V_{max}}$. Based on the data, the Michaelis-Menten parameters calculated are as follows:

$$V_{max} = 17.57 \frac{mMol}{L * min}$$

$$K_m = 9.97 mM$$

1.4 Discussion

Possible sources of systematic error in this experiment include the following: improper calibration of pipette, glucose analyzer, and weighing instrumentation, contamination of stock chemicals, and dirty glassware. Random errors are likely due to equipment malfunction such as a faulty electrode connection in the glucose analyzer. The literature value of the Michalis-Menten constant is 25mM, which is different from the experimental value [3]. This, however, is to be expected as the process conditions and the enzyme concentration are likely different.

1.5 Conclusions and Future Work

The methods outlined in this chapter are a robust way to quantify the enzyme kinetics of invertase. Experiments can be repeated at different enzyme or sucrose concentrations, as well as at different temperatures, to show the affect these parameters have on the enzyme kinetics. From start to finish the entirety of this experiment can be completed in two to three hours including solution prep and data collection.

CHAPTER 2

SEPARATION OF FRUCTOSE AND GLUCOSE BY ION EXCHANGE CHROMATOGRAPHY

The objective of this work is to determine the effect of temperature, flowrate, pH, and resin particle size on calcium cation exchange chromatography for separating a mixture of fructose and glucose. The basis for calcium cation exchange chromatography is interactions between components in the mobile phase and the cation exchange resin, or stationary phase. Separating the monosaccharides glucose and fructose is essential for the production of high fructose corn syrup in the food industry. Most methods of sugar separation are very costly including reverse osmosis [4], zeolite adsorption [5], complexing the sugars using borates in electrodialysis [6], and using liquid membranes [7]. Chromatographic separation with a cation exchange resin was investigated in this report.

2.1 Introduction

There are a variety of chromatographic techniques employed to separate biomolecules. Each technique relies on physical differences in properties such as size or charge between biomolecules as the basis for separation. Table 2.1 summarizes which technique should be used for each property. This thesis will primarily focus on ion exchange chromatography.

Table 2.1: *Chromatography Techniques [8]*

Property	**Technique**
Size	Size exlusion chromoatography (SEC), Gel filtration (GF)
Hydrophobicity	Hydrophobic interaction chromatography (HIC)
	Reverse phase chromatography (RPC)
Charge	Ion exchange chromatography (IEX)
Biorecognition (ligand specificity)	Affinity chromatography (AC)
Isoelectric point (PI)	Chromatofocusing (CF)

The basis of separation for ion exchange chromatography is charge; molecules are separated according the strength of their overall ionic interaction with a solid phase material. In cation exchange chromatography, the solid phase resin has a negative charge, whereas in anion exchange chromatography, the resin has a positive charge [9]. The naming convention "ion exchange chromatography" is somewhat deceiving in this case. There is no ion exchange between the resin and the sugars involved. The "exchange" has already happened by attaching the Ca^{2+} ions to the resin. Instead, the purpose of the resin is to adsorb, and therefore slow, fructose as it moves down the column. Both fructose and glucose form a complex with the calcium ions attached to the resin. However, the complex that calcium ions form with fructose is stronger than the complex formed with glucose. The formation of these complexes causes fructose to take longer to elute out of the column; this is the basis of separating the two sugars. In other words fructose has a higher *affinity* to the calcium ion than glucose does. This process was first patented by Neuzil and Priegnitz [10].

2.2 Experimental Methods

A Spectrum Labs (Houston, TX) jacketed glass column was used for the chromatography with a length of 30 cm and ID of 1.5 cm. A loading reservoir was used to fill the column with degassed buffer prior to be packed with one of two Dowex monosphere 99 calcium, Ca^{2+}, gel- type resins (Dow Chemical Company). The resin with a particle size of either 280 μm or 220 μm was allowed to settle overnight before being flushed with three column volumes of buffer by gravity. The plunger attachment was positioned just above the top of the resin and secured in place. A Scientific Systems Incorporated (State College, PA) HPLC metering pump was then used to pump buffer up the column, in the reverse direction, to remove air from the tubing and six-way sample valve. With the air removed from the system, the pump was reconnected to the sample valve, pumping buffer into the top of the column. Samples were injected into the 1.2 mL sample loop using the six-way valve. The column effluent was collected by a Spectrum Labs CF-2 fraction collector in

one minute increments. Fractions were analyzed with a Vee Gee Scientific (Kirkland, WA) refractometer and a VacciXCell (Singapore) glucose analyzer. The glucose, fructose, and calcium chloride were laboratory grade from Sigma-Aldrich (St. Louis, MO). The materials and equipment used in this lab are listed in Appendix A in Table A.2 and Table A.1 respectively.

2.3 Results

The refractive index of the effluent as well as the concentration of glucose in each fraction were measured every minute. UV absorption for sugars is impractical, as sugars can only be detected near the 190 to 195 nm [11]. It is possible to use UV detection, but this requires a color reaction such as Benedict's reagent [11]. Therefore, refractive index was chosen as a detection method. The concentration of fructose was found by first converting the glucose concentration to a refractive index value using Equation 2.1

$$RI = Glucose\ Concentration(mg/mL) * 0.0894 \tag{2.1}$$

where RI is the refractive index. Then, subtracting the calculated refractive index value from the total RI curve and converting that value to fructose concentration using Equation 2.2.

$$RI = Fructose\ Concentration(mg/mL) * 0.0862 \tag{2.2}$$

The total refractive index, glucose concentration, and fructose concentration were used to plot chromatograms and calculate the separation factor using Equation 2.3.

$$\alpha = \frac{K_2}{K_1} = \frac{V_2 - V_0}{V_1 - V_0} = \frac{t_2 - t_0}{t_1 - t_0} \tag{2.3}$$

where t_0, which is the total void volume equal to 12 minutes, was found by injecting calcium chloride into the column and measuring the time it takes to elute. This value depends on the experimental apparatus.

2.3.1 Effect of Temperature

The chromatograms for 5°C, 25°C, and 45°C at a neutral pH and same flowrate are depicted in Figure 2.1, 2.2, and 2.3 respectively. Running the column at a lower temperature produced better results and therefore a higher separation factor as seen in Table 2.2.

Table 2.2: *Separation factor at varied temperatures*

Temperature (°C)	Separation Factor (α)
5°C	1.67
25°C	1.50
45°C	1.25

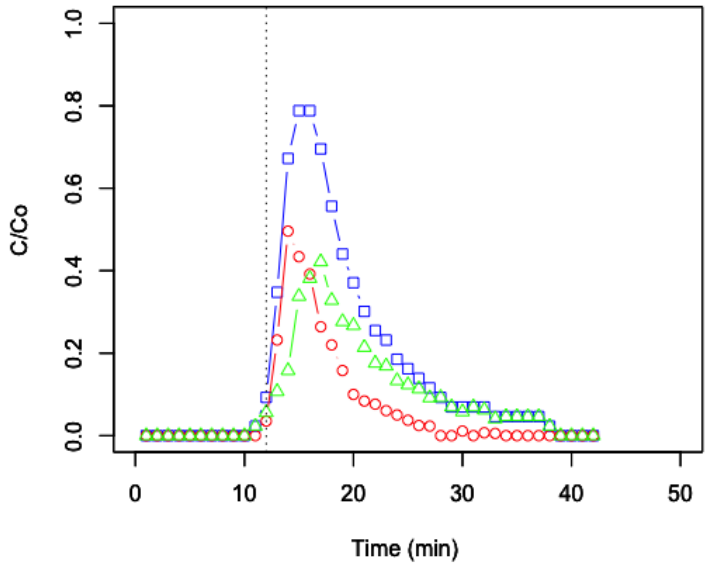

Figure 2.1: *Normalized concentration as a function of time (min) for total sugar concentration (blue squares), glucose (red circles), and fructose (green triangles). V = 2.5 mL/min, pH = 7, T = 5°C, t_0 depicted as a horizonal line at t=12min*

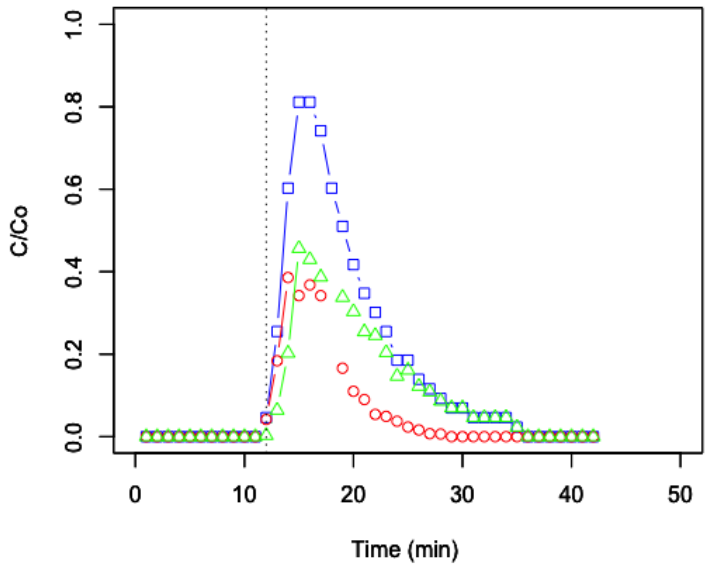

Figure 2.2: *Normalized concentration as a function of time (min) for total sugar concentration (blue squares), glucose (red circles), and fructose (green triangles). V = 2.5 mL/min, pH = 7, T = 25°C, t_0 depicted as a horizonal line at t=12min*

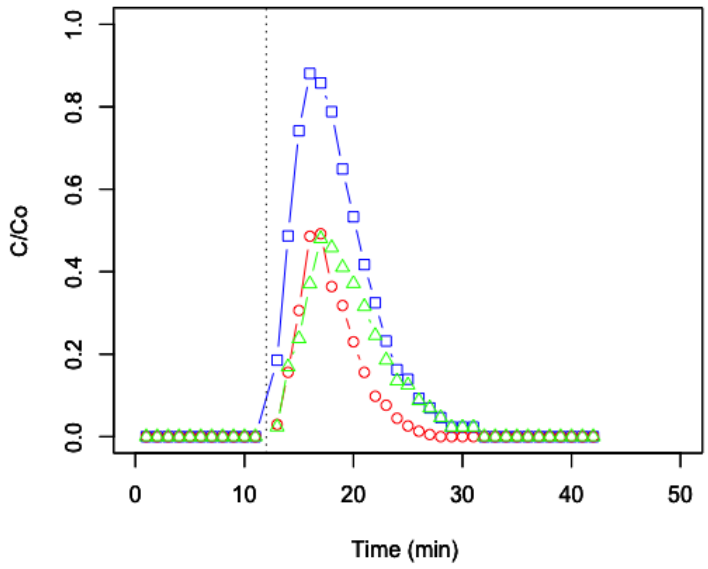

Figure 2.3: *Normalized concentration as a function of time (min) for total sugar concentration (blue squares), glucose (red circles), and fructose (green triangles). V = 2.5 mL/min, pH = 7, T = 45°C, t_0 depicted as a horizonal line at t=12min*

2.4 Discussion

Possible sources of systematic error in this work include the following: improper calibration of temperature, refractometer, pump, and weighing instrumentation, bubble formation, contamination of stock chemicals, and leaks in the apparatus. Random errors are likely due to equipment malfunction such as a power surge and nonuniform pump output.

2.5 Conclusions and Future Work

The methods outlined in this chapter are a strong indication that ion exchange chromatography can be used to separate fructose and glucose. Future work should include investigating zeolite and different strong cation exchange resins.

REFERENCES

[1] F. H. C. Kelly and D. W. Brown, "Thermal decomposition and colour formation in aqueous sucrose solutions," *Sugar Technology Reviews*, vol. 6, pp. 1–47, 1 1978.

[2] V. Buddolla, *Recent Developments in Applied Microbiology and Biochemistry*. Academic Press, 2019.

[3] *Invertase from baker's yeast (s. cerevisiae)*, I4504, Sigma-Aldrich, 2013.

[4] S. S. Kim, H. N. Chang, and Y. S. Ghim, "Separation of fructose and glucose by reverse osmosis," *Ind. Eng. Chem. Fundam.*, vol. 24, pp. 409–412, 1985.

[5] Y. L. Cheng and T. Y. Lee, "Separation of fructose and glucose mixture by zeolite y," *Biotechnology and Bioengineering*, vol. 40, no. 4, pp. 498–504, 1992.

[6] D. Langev, M. Metayer, M. Labbe, and C. Chappey, "Electromembrane process for sugar separation: A preliminary study," *Russ. J. Electrochem*, vol. 32, no. 2, 241–247, 1996.

[7] M. Di Luccioa, B. D. Smith, T. Kida, C. P. Borges, and T. L. M. Alves, "Separation of fructose from a mixture of sugars using supported liquid membranes," *Journal of Membrane Science*, vol. 174, pp. 217–224, 2000.

[8] *Size exclusion chromatography: Principles and methods*, GE Healthcare Life Sciences, 2014.

[9] R. G. Harrison, P. Todd, S. R. Rudge, and D. P. Petrides, *Bioseparations Science and Engineering*. Oxford University Press, 2003.

[10] R. W. Neuzil and J. W. Priegnitz, *Process for separating glucose from fructose by selective adsorption*, US Patent 4,349,668, 1982.

[11] *Methods for separating sugars*, Shimadzu, 2019.

Appendices

APPENDIX A

EXPERIMENTAL MATERIALS AND EQUIPMENT

Table A.1: *Equipment*

Equipment	**Manufacturer**	**Model**
Glucose Analyzer	VacciXCell (Singapore)	GlucCell
Micropipette	Eppendorf (Hamburg, Germany)	2.5-5000 μL
Vortex Machine	Scientific Industries (Bohemia, NY)	Vortex Genie-1
Balance	Mettler Toledo (Columbus, OH)	XS203S
Fraction Collector	Spectrum labs (Houston, TX)	CF-2
HPLC Reciprocating Pump	Teledyne Scientific Systems (Pittsburgh, PA)	Series I
Circulating bath	Neslab (Portsmouth, NH)	Endocal RTE-5B
Chromatography Column	Spectra Labs (Houston, TX)	30cm
Refractometer	Vee Gee Scientific (Vernon Hills, IL)	MDX Series

Table A.2: *Materials*

Material	Supplier	Catalog number	Lot number
DI water	n/a	n/a	n/a
Invertase	Sigma-Aldrich	I4504-250MG	SLBV3715
Sucrose	Sigma-Aldrich	S9378-1KG	SLBW4014
Fructose	Sigma-Aldrich	F3510-500G	SLBZ1343
Glucose	Sigma-Aldrich	G8270-1KG	108K0031
Na_2CO_3	Sigma-Aldrich	222321-1KG	025K01271
Dowex™ Monosphere™	Dow Chemical	99CA/220	1567076
Dowex ™ Monosphere™	Dow Chemical	99CA/280	1567078
Kimwipes	Fisher Scientific	06-666	n/a

Figure A.1: *GlucCell Glucose Analyzer (© Copyright Esco VacciCell)*

Figure A.2: *Reciprocating HPLC metering pump (© Copyright Teledyne Scientific Systems, Inc.)*

Figure A.3: *Refractometer (© Copyright Vee Gee Scientific, Inc.)*

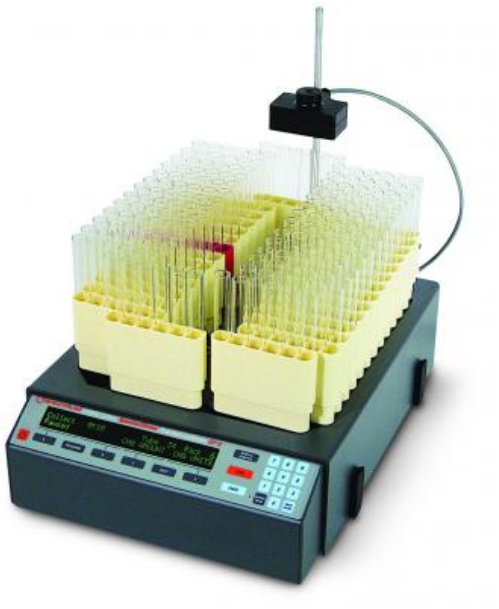

Figure A.4: *Fraction Collector (© Thermo Fisher Scientific)*

Figure A.5: *Fraction Collector (© BioRad)*

Figure A.6: *Vortex Genie I (© Scientific Industries)*

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